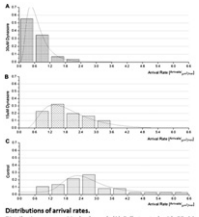
2228-Pos Board B214
Quantification and Manipulation of MHC-I Delivery to Cell Plasma Membrane
Daniel Blumenthal.
An invaded cell reports to the immune system it has been compromised by a process involving clustering of Major Histocompatibility Complex Class I (MHC-I) protein on the cell membrane surface. In our work we concentrated on the mechanism by which MHC-I is trafficked to the cell membrane; although predictions existed, the dynamics of the reporting processes were not fully understood for the past 15 years. Using Total Internal Reflection Fluorescence Microscopy (TIRFM) in combination with sophisticated image analysis procedures, we quantified the rate of delivery of MHC-I to the plasma membrane. Additionally, we developed a simple way to control (reduce) the delivery rate. Furthermore, we provide supporting evidence for the fact that MHC-I are primarily trafficked in Clathrin-coated vesicles.


Distributions of arrival rates.
Distributions are positively skewed. (A) Cells treated with 30μM dynasore distribution most probable number is $0.3X10^{-3}$ [$\mu m^{-2}min^{-1}$] and mean of $0.68X10^{-3}$ [$\mu m^{-2}min^{-1}$] arrivals (B) Cells treated with 15μM Dynasore distribution most probable number is $1.5X10^{-3}$ [$\mu m^{-2}min^{-1}$] and mean is $1.86X10^{-3}$ [$\mu m^{-2}min^{-1}$] arrivals (C) Untreated cells distribution most probable number is $2.7X10^{-3}$ [$\mu m^{-2}min^{-1}$] and mean is $2.67X10^{-3}$ [$\mu m^{-2}min^{-1}$] arrivals.

Membrane Transporters & Exchangers II

2229-Pos Board B215
Transporting Mechanisms of Sodium-Dependent Secondary Membrane Transporters: Insights form Computational Simulations
Chunfeng Zhao, Javier Cuervo, Sergei Y. Noskov.
Recent progress in crystallographic studies of sodium-coupled secondary transporters has revealed striking similarities in the structural organization of ion and solute binding motifs and a well-conserved inverted-repeat topology between proteins from several gene families. Molecular Dynamics and free energy simulations are applied to study the mechanisms of selective binding of ion and substrate in LeuT, vSGLT, and Mhp1. We found that water molecules accessed from periplasm and cytoplasm to the binding sites play an important role in affinity and selectivity of the ion/substrate binding. String method is applied to study the transition from outward-facing state to inward-facing state, providing a minimal-free energy pathway of the transition. These studies identify the sequence of ion and substrate binding/releasing associated with the transporting cycle and provide microscopic mechanical mechanisms of gating.

2230-Pos Board B216
On How the Conformational Cycle of the AcrB Efflux Pump is Coupled to Proton Translocation: A Theoretical Study Based on High-Resolution Structural Data
Claudio Anselmi, Wenchang Zhou, Kay Diederichs, Klaas M. Pos, José D. Faraldo-Gómez.
The AcrA/AcrB/TolC multidrug efflux pump confers *Escherichia coli* with antibiotic resistance by sequestering toxic compounds found within the periplasm and inner membrane and extruding them into the extracellular space. The AcrB trimer is the central component of this efflux complex; anchored in the inner membrane, it forms an asymmetric assembly that undergoes a conformational cycle in which each protomer adopts three different structures. As a result, substrates bound in the periplasmic domain of AcrB are projected into the TolC channel, which reaches beyond the outer membrane. Crucially, the conformational cycle within AcrB is driven by the translocation of protons down the gradient sustained by the inner membrane, through a mechanism that has not been characterized so far.
Here, we investigate this microscopic mechanism through atomistic free-energy molecular dynamics simulations and electrostatic calculations, based upon novel high-resolution structural data for wild-type and mutagenized AcrB. Specifically, we assess the events associated with binding and release of protons within the membrane domain, and determine the mechanism by which these events are coupled to the reorganization of key transmembrane helices within each protomer. This investigation reveals how proton translocation influences both local and remote interactions within the protein, thereby modulating its structure.

2231-Pos Board B217
Mechanism of Intracellular Gating in the Glutamate Transporter
Zhijian Huang, Emad Tajkhorshid.
Glutamate transporters (GlTs) are membrane proteins that regulate and remove synaptically released neurotransmitter glutamate, and maintain normal excitatory synaptic transmission. The recently solved structure of inward-facing GltPh, a GlT homologue revealed an occluded state with the substrate and two Na+ ions (Na1 and Na2) bound.
The inward-facing and outward-facing structures of GltPh have put forward a molecular mechanism by which the transporter mediates Na+-coupled substrate uptake. However, the molecular nature of the intracellular gate and the mechanism of gating are still unknown. Furthermore, the mechanism of release of the substrate and co-transported Na+ ions from their intracellular binding sites remains elusive. We have investigated the transporter's dynamics and the coupling between substrate and Na+ ions using an extensive set of molecular dynamics simulations of membrane-embedded model of inward-facing GltPh in various bound states. The results suggest that the helical hairpin HP1 plays the key role of the intracellular gate for the substrate-binding site, and that the opening and closure of the gate is controlled by the Na+ ion in the Na1 site. The Na+ ion in the Na2 site was found to be the first to be released from the inward-facing occluded state and can diffuse into the cytoplasmic solution through the attraction of highly conserved residue Ser65 in TM2. Moreover, upon unbinding of the Na+ ion in the Na1 site, the substrate was observed to completely unbind from the binding site and diffuse into the cytoplasmic solution in our equilibrium simulations along the opening of the intracellular gate HP1. Based on the simulations, we propose that the two structurally resolved Na+ ions release into the cytoplasm froe the inward-facing Gltph before the substrate.

2232-Pos Board B218
Looking for the Inward Facing State of Glutamate Transporters
Xiaoyu Wang, Marta Rodriguez, **Peter Larsson**.
A recent crystal structure of the bacterial glutamate transporter homologue GltPh (Reyes et al., 2009) was interpreted as the inward facing state of glutamate transporter. Comparing this inward facing structure to the earlier outward facing structures of GltPh suggested that glutamate transporters undergo a large conformational change between these two states. In contrast, earlier FRET experiments from our group (Koch and Larsson, 2005) suggested that the mammalian glutamate transporter EAAT3 does not undergo large conformational changes during the uptake cycle. We here tested the proposed inward facing state of glutamate transporters using different FRET techniques under different ion and substrate conditions that, we assume, forces EAAT3 to mainly occupy the inward facing state or the outward facing state.

Ryanodine Receptors

2233-Pos Board B219
Ryanodine Receptor is a Magnesium Channel: Consequences of Selectivity on Physiology
Dirk Gillespie.
The ryanodine receptor (RyR) releases Ca^{2+} out of the sacroplasmic reticulum (SR) and is therefore thought of a calcium channel. However, the most abundant divalent cation is Mg^{2+} (present at ~1 mM both in the cytoplasm and the SR) and RyR has a very high affinity for symmetrically applied Mg^{2+}. This is seen in single-channel recordings when even 1 mM of symmetric Mg^{2+} reduces the current from 10 mM luminal Ca^{2+} by ~25%. Here it is shown why changes in $[Mg^{2+}]$ (e.g., due to ischemia or exercise) disproportionately affect unitary Ca^{2+} current, in addition to changing RyR open probability. A recent model of ion permeation through RyR is used to first describe the mechanism behind this high Mg^{2+} affinity and RyR's weak K^+ affinity and then analyze their effect on Ca^{2+} release. Loosely speaking, $[K^+]$ defines the SR membrane potential and $[Mg^{2+}]$ defines unitary Ca^{2+} current for a fixed SR membrane potential and SR Ca^{2+} load. Counterintuitively, while K^+ occupancy in the pore is low, K^+ still provides the vast majority of countercurrent during Ca^{2+} release. Moreover, the opposite is true for Mg^{2+}; unless $[Mg^{2+}]$ is unusually high, Mg^{2+} provides little countercurrent despite having the largest number of ions in the pore.

2234-Pos Board B220
Ouabain is a Pharmacomimic of Mutant RyR2 Ca2+ Release Dysfunction but is not a Serum-Borne Trigger of CPVT
Steven R. Barberini-Jammaers, Nicole C. Silvester, Ben H. Maskrey, Gabriele Banci, Raffaella Bloise, Mark C. Bagley, Valerie B. O'Donnell, Carlo Napolitano, Silvia G. Priori, F. Anthony Lai, **Christopher H. George**.
Mutation-linked RyR2 Ca2+ handling dysfunction is an intracellular driver for stress-evoked arrhythmias. Disease pathogenesis links augmented β-adrenergic stimulation to the acute dysfunction of mutant RyR2. However, the incomplete efficacy of β-blockade in clinical and experimental scenarios suggests that other serum-borne factors, in addition to catecholamines, contribute to Ca2+